REMARKS

Claims 1-9 and 11-18 are pending. No new matter has been added by way of the above amendments. For example, claim 1 has been amended to recite the subject matter of originally filed claim 8 and previously pending claim 10. Claim 6 has been amended to remove the recitation of alkyltrimethyl ammonium salts. Lastly, claim 8 has been amended to be consistent with the amendments to claim 1. Accordingly, no new matter has been added.

Applicants further submit that no new issues have been raised by way of the present submission which would require additional search and/or consideration on the part of the Examiner. For instance, claim 1 has been amended to recite the subject matter of claims 8 and 10 which were already presumably searched and considered. Thus, the inclusion of this subject matter in claim 1 does not present any new issues for the Examiner. Moreover, the amendment to claim 6 removes a member of the Markush group and does not create a new issue. Claim 8 does not raise new issues since it merely has been amended to reflect the amendments made in claim 1. Accordingly, no new issues have been raised which would require additional search and/or consideration on the part of the Examiner.

In view of the following remarks Applicants respectfully request that the Examiner withdraw all rejections and allow the currently pending claims.

Issues Under 35 U.S.C. §103(a)

The Examiner has rejected claims 1 and 8-10 under 35 U.S.C. §103(a) as being obvious over Aoki in view of Voet. The Examiner has also rejected claims 2-7 under 35 U.S.C. §103(a) as being obvious over Aoki in view of Voet and Lapicola. Applicants respectfully traverse these rejections.

The Present Invention and Its Advantages

The present invention relates to a method of immunologically measuring the human medullasin content in blood. The present method involves a first step in that the leukocytes in the blood samples are lysed by contacting with a specific aqueous liquid mixture according to either of limitations (i) and/or (ii) according to claim 1. Next, the present invention allows for determining the content of human medullasin in this blood sample. The step of determining the content of human medullasin is defined in step (b) of claim 1. This step clarifies that the immunoassay of the present invention relates

to measuring the human medullasin content in the blood sample using a monoclonal antibody highly reactive with the medullasin. The make up of the present invention allows for the collection of accurate data of medullasin content in the blood sample.

Claims 1 and 8-10

The Examiner rejects claims 1 and 8-10 as being obvious over Aoki in view of Voet. Aoki, however, merely discuss an enzyme immunoassay for quantitatively measuring medullasin in a blood sample by using a polyclonal antibody. Aoki is silent regarding dissolution treatment of the granulocytes.

Aoki disclose an incubation step in which the IgG-coated polystyrene balls are shaken for 2 hours in the blood diluted with a 0.1 mol/l sodium phosphate solution as the buffer to 0.15 ml in the final stage, at pH 6.5 and 37°C. This step is for the reaction between the polyclonal antibody and medullasin antigen, and the conditions therefore are not in agreement with the granulocyte-lysing conditions for the present invention. In this case, the concentration of the soldium phosphate solution is 0.01 mol%. The present invention dissolves the granulocytes by the aid of an aqueous liquid contacting the blood sample at an osmotic pressure of 250mOsm/kg H_2O or less or 310 mOsm/kg H_2O

or more, and a solute, e.g., sodium phosphate, present at 0.05 mol% or more (preferably 0.1 mol% or more) or 0.005 mol% or less (refer to page 7 bottom line - page 8, line 5). By contrast, Aoki use 0.01 mol% of sodium phosphate buffer. This condition is outside of the granulocyte-lysing conditions of the present invention.

Therefore, the step of dissolving the granulocytes in the blood sample is not inherent in method of Aoki. Also, Aoki neither disclose nor suggest the dissolving step of the present invention.

Voet discloses isolation of protein located in the cytosol of cells by osmosis. However, Voet merely disclose isolation in general of protein located in the cytosol of cells, and is completely silent on the conditions under which the granules are dissolved in the blood, in particular for medullasin.

The treatment of dissolving the granulocytes may be effected for the present invention by the aid of refined water (osmotic pressure: 0mOsm/kg•H_2O) as described in Example 2 in the specification of the present invention. The variation coefficients observed in this example are 1.7 and 1.8, as shown in Table 1. These results compare with the coefficients of 10.1 and 10.4 observed in Comparative Example 1, as shown in Table 2.

These greatly different results clearly indicate that the present invention brings a notably different result.

Under conditions similar to Comparative Example 1, Aoki use blood diluted by using 0.1 mol/l phosphate buffer, and then determine the content of the medullasin in the granulocytes. However, as the lysing conditions were not fully met, there were large variations in the measurements and the measured data of poor reproducibility was obtained.

Based on these distinctions alone, the Examiner has failed to present a *prima facie* case of obviousness.

However, the present invention further differs in that the present invention uses a monoclonal antibody as the antibody to be reacted with the medullasin antigen in the enzyme immunoassay for measuring the medullasin. By contrast, Aoki measure the medullasin by a polyclonal antibody (page 195 of Aoki).

A polyclonal antibody is completely different from a monoclonal antibody. Aoki require 18 hours to measure the medullasin (a total time required for the incubations is 18 hours which consists of 2 hours of the first incubation as shown on the bottom line of page 195 and 16 hours of the second incubation as shown on line 3 of page 196. In contrast, the present invention requires only 1 hour (30 minutes for the first

incubation and 30 minutes for the second incubation as shown in Example 2.) to measure the medullasin.

A polyclonal antibody is a mixture of antibodies of widely varying reactivity, from high to low level. Measurement of the medullasin by the aid of a polyclonal antibody requires a long reaction time, because of a high content of low-reactivity antibodies in the polyclonal antibody. By contrast, the monoclonal antibody of the present invention is composed of a single antibody of very high reactivity. This consequently helps determine the medullasin content in a very short time.

The difference between a polyclonal and monoclonal antibody in reactivity depends greatly on type of antigen with which it reacts. A monoclonal antibody shows much higher reactivity than a polyclonal antibody with the medullasin, which is specific to the monoclonal antibody. Neither Aoki nor Voet suggest or disclose this specificity.

Therefore, the monoclonal antibody showing very high reactivity with the medullasin is a novel and non-obvious one, and thus, the immunoassay system that uses such an antibody is also novel and non-obvious. Moreover, the present invention further has unexpected results since the immunoassay system of

the present invention is functionally superior to that of Aoki et al as discussed above.

In summary, the Aoki and Voet references fail to suggest or disclose the specific lysing step and lysing buffers according to the present invention. Moreover, neither Aoki nor Voet suggest or disclose the use of the specific monoclonal antibody according to step (b) of independent claim 1. Accordingly, the Examiner has failed to establish a *prima facie* case of obviousness. Moreover, even if, arguendo, the Examiner has hypothetically established a *prima facie* case of obviousness, unexpected results exist with respect to the present invention. For instance, as discussed above the present invention is functionally superior to the primary reference of Aoki. These results are fully unexpected.

In order to aid the Examiner in the analysis of the relationship between the present invention and Aoki, Applicants have attached hereto a comparison. This comparison does not present any new experimental evidence but merely provides a direct comparison between the methods of the present invention and the methods of Aoki.

In summary, the Examiner's rejection under 35 U.S.C. 103(a) based upon Aoki in view of Voet of claims 1 and 8-10 is moot.

Reconsideration and withdrawal thereof are respectfully requested.

The Examiner has also rejected claims 2-7 under 35 U.S.C. §103(a) as being obvious over Aoki in view of Voet and Lapicola. Applicants traverse this rejection. As indicated above, significant deficiencies exist in the Examiner's rejection with respect to claims 1 and 8-10 regarding Aoki and Voet. Lapicola fails to cure any of these deficiencies. Accordingly, this rejection is moot.

As an additional distinction, Applicants point out that claim 6 no longer recites the organic cell dissolving agent "alkyltrimethyl ammonium salts". Accordingly, the use of the Lapicola reference is further moot in view of this amendment. Reconsideration and withdrawal of this rejection is respectfully requested.

In view of the above remarks, Applicants respectfully request that the Examiner withdraw all rejections and allow the currently pending claims. That is, Applicants have shown that the present invention differs from the disclosures of Aoki, Voet and Lapicola. Accordingly, a Notice of Allowability directed to claims 1-9 and 11-18 is respectfully solicited.

If the Examiner has any questions or comments, please contact Craig A. McRobbie (Reg. No. 42,874) at the offices of Birch, Stewart, Kolasch & Birch, LLP.

Pursuant to 37 C.F.R. § 1.17 and 1.136(a), Applicants respectfully petition a two (2) months extension of time for filing a response in connection with the present application. The required fee of $400.00 is attached hereto.

Applicants have attached hereto a marked up version of the claims to show the changes made for the Examiner's convenience.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. §§ 1.16 or 1.17; particularly, extension of time fees.

Respectfully submitted,

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Attachment: Version with Markings to Show Changes Made

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE CLAIMS:

Claim 10 has been cancelled.

The claims are amended as follows:

1. (Twice Amended) A method of immunologically measuring the human medullasin content in blood comprising the following steps (a) and (b):

(a) breaking up the leukocytes in a blood sample by contacting said blood sample with the following aqueous liquids (i) or (ii) or an aqueous liquid mixture of (i) and (ii)

(iii) an aqueous liquid having an osmotic pressure of 250mOsm/kg•H_2O or less or an aqueous liquid having an osmotic pressure of 310mOsm/kg•H_2O or more;

(iv) an aqueous liquid comprising a hemolysate; and

(b) immunologically determining [the amount of human medullasin released into said blood sample from the leukocytes broken up in said step (a) using an anti-human medullasin antibody] content of human medullasin in said blood sample by a

method comprising contacting the blood sample containing said human medullasin released from the leukocytes broken up in said step (a) with an anti-human medullasin monoclonal antibody immobilized to an insoluble carrier in the presence of a labeled anti-human medullasin monoclonal antibody to form a sandwich complex and to capture the human medullasin on a labeled immuno complex by an antigen-antibody reaction, and then determining the amount of activity of the label material in said complex.

6. (Amended) The method of immunologically measuring the human medullasin content in blood according to claim 5, wherein said aqueous liquid (ii) is an aqueous solution of at least one type of hemolysate selected from the group consisting of higher fatty acid salts, alkylaryl sulphonates, alkyl sulphonates, alkyl sulphate ester salts, alkyl pyridinium salts, [alkyltrimethyl ammonium salts,] polyoxyethylene alkylphenyl ethers, polyoxyethylenealkylethers, polyoxyethylene sorbitan fatty acid esters and alkyl betaines.

8. (Twice Amended) The method of immunologically measuring the human medullasin content in blood according to claim 1 wherein said step (b) of immunologically determining the content of human medullasin in said blood sample comprises [contacting the blood sample containing said human medullasin released from the leukocytes broken up in said step (a) with an anti-human medullasin antibody immobilized to an insoluble carrier in the presence of a labelled anti-human medullasin antibody to form a sandwich complex and to capture the human medullasin on a labelled immuno complex by an antigen-antibody reaction, and then determining the amount of activity of the label material in said complex] sandwiching said human medullasin in said blood sample between an anti-human medullasin antibody immobilized to an insoluble carrier and a labeled anti-human medullasin antibody to form a complex by an antigen-antibody reaction, and determining the amount of label in said complex.

Comparison with the present invention and Aoki et al

	The present invention Example 2	Comp. Example 1	Aoki et al
I	10μl of each sample of a normal individual and from a patient was added to 2ml of distilled water (0mOsm/kg·H_2O) and mixed using a Voltex mixer to obtain sample solutions.	10μl of each sample of a normal individual and from a patient was added to 2ml of PBS (pH7.4) (about 290mOsm/kg·H_2O) and mixed using a Voltex mixer to obtain sample solutions.	Not disclosed
II	10μl thereof was then added to test tubes, and was diluted by the addition of 390μl of PBS solution (pH7.4) containing 2% BSA.	10μl thereof was then added to test tubes, and was diluted by the addition of 390μl of PBS solution (pH7.4) containing 2% BSA.	Polystyrene balls coated with IgG were incubated with various amounts of medullasin or peripheral blood diluted with 0.1mol/l sodium phosphate buffer, pH6.5, at 37°C for 2h with shaking in a final volume of 0.15ml.
III	Beads having mouse anti-human medullasin monoclonal antibody (3FO3) immobilized thereto were added one each to these test tubes and incubated at 37°C for 30min.	Beads having mouse anti-human medullasin monoclonal antibody (3FO3) immobilized thereto were added one each to these test tubes and incubated at 37°C for 30min.	
IV	After removal of the solutions in the test tubes by aspiration, they were washed with physiological saline solution, the test tubes were filled with 400μl of PBS solution containing 2% BSA and containing HRP-labelled mouse anti-human medullasin monoclonal antibody (2EO4) in a concentration of 0.2μg/ml followed by incubation at 37°C for 30min.	After removal of the solutions in the test tubes by aspiration, they were washed with physiological saline solution, the test tubes were filled with 400μl of PBS solution containing 2% BSA and containing HRP-labelled mouse anti-human medullasin monoclonal antibody (2EO4) in a concentration of 0.2μg/ml followed by incubation at 37°C for 30min.	After incubation polystyrene balls were washed twice with 1ml of buffer A without NaN_3 and incubated with 50ng of the conjugate in a final volume of 0.15ml A without NaN_3 at 25°C for 16h with shaking.
V			